J. Parasitol., 78(2), 1992, p. 281–287


# DETECTION AND PRELIMINARY CHARACTERIZATION OF *TAENIA SOLIUM* METACESTODE PROTEASES

**A. Clinton White, Jr., Jose Luis Molinari*, Anilkumar V. Pillai, and Ajay A. Rege†**

Departments of Internal Medicine, Family Medicine, and Microbiology and Immunology, Baylor College of Medicine, One Baylor Plaza, Houston, Texas 77030

ABSTRACT: The metacestode of *Taenia solium* persists for years in the human central nervous system. As proteolytic enzymes play an important role in the survival of tissue helminths, we examined extracts of *T. solium* metacestodes for proteolytic activity using 9 synthetic peptide substrates and 3 proteins (hemoglobin, albumin, and immunoglobulin G). The proteolytic enzymes were classified based on their inhibitor profiles. At neutral pH, aminopeptidase (arginine-7-amino-4-trifluoromethylcoumarin) and endopeptidase (benzyloxy-carbonyl-glycine-glycine-arginine-7-amino-4-trifluoromethylcoumarin) substrates were cleaved. Hydrolysis of both substrates was inhibited by chelating agents, which inhibit metalloproteases. Peak activity with both substrates eluted in gel filtration fractions corresponding to a molecular weight of about 104 kDa. Cysteine protease activity was identified, which cleaved benzyloxy-carbonyl-phenylalanine-arginine-7-amino-4-trifluoromethylcoumarin (Z-Phe-Arg-AFC) and hemoglobin. Cleavage of Z-Phe-Arg-AFC was maximal at acid pH, was stimulated by thiols, and was inhibited by leupeptin and Ep459. Peak cysteine protease activity eluted in gel filtration fractions corresponding to a molecular weight of 32 kDa. Aspartic protease activity was identified by specific inhibition with pepstatin of acid digestion of hemoglobin and immunoglobulin G. Immunoglobulin digestion occurred at acid pH, with preferential degradation of the heavy chain. Upon gel filtration chromatography, the aspartic protease activity eluted as a broad peak with maximal activity at about 90 kDa. No serine protease activity was detected. None of the parasite enzymes digested albumin. Proteolytic enzymes of *T. solium* may be important for parasite survival in the intermediate host, by providing nutrients and digesting host immune molecules.

Human neurocysticercosis is caused by infection of the central nervous system with the metacestode of *Taenia solium*. In the normal life cycle, *T. solium* eggs hatch in the gut of the intermediate host. After the larvae migrate to the muscles and central nervous system, they transform into the metacestode stage over a period of months. To complete the life cycle, the metacestode must remain viable in tissues until ingested by the definitive host. Clinical studies suggest that the metacestode can survive in the human nervous system for years prior to onset of disease (Dixon and Lipscomb, 1961; Earnest et al., 1987; Flisser, 1988).

In order for the metacestode to survive, it must have access to nutrients and must also avoid destruction by the host. Proteolytic enzymes play an important role in the survival of other tissue-dwelling helminths (reviewed by McKerrow [1989]). For example, a metalloproteinase from hookworm larvae may aid in penetration of the host skin and may aid in parasite nutrition by digesting host fibrinogen (Hotez and Cerami, 1983; Hotez et al., 1985, 1990). The serine proteinase from *Schistosoma mansoni* larvae may modulate host defenses by cleaving host immunoglobulin and by releasing immunomodulating peptides derived from host molecules (Auriault et al., 1980, 1981; Verwaerde et al., 1988). The adult worm cysteine proteinases from *S. mansoni* digest hemoglobin, a major parasite nutrient (Zerda et al., 1988). Here we report initial studies on the proteolytic activity of *T. solium* metacestodes.

## MATERIALS AND METHODS

### Parasites

Metacestodes of *T. solium* were dissected from the muscle of a naturally infected pig and lyophilized. Extracts were prepared by suspending metacestodes (20 mg dry weight/ml) in either 200 mM sodium citrate, pH 4.9, or 200 mM phosphate-buffered saline, pH 7.5 (PBS), and grinding them with a Teflon–glass homogenizer. Particulate material was removed by centrifugation (10,000 *g*, 25 min, 4 C), and the supernatant fraction (S1) was assayed for enzyme activity. Protein content was assayed by the method of Bradford (1976).

### Protease assays with peptide substrates

Synthetic peptide substrates were purchased from Enzyme Systems Products (Livermore, California). The peptide substrates and their usual specificities are as follows: benzyloxy-carbonyl-phenylalanyl-arginyl-7-amino-4-trifluoromethylcoumarin (Z-Phe-Arg-AFC) (cysteine and serine protease); Z-Arg-AFC, Z-Gly-Gly-Arg-AFC, succinyl-Ala-Ala-Pro-Phe-AFC, methoxy-

Received 13 August 1991; revised 17 October 1991; accepted 17 October 1991.

* Departamento de Microbiologia, Instituto de Fisiologia Cellular, Universidad Nacional Autonoma de Mexico, Apartado 70-242, 04510 Mexico, D.F., Mexico.

† Department of Biochemistry, Baylor College of Medicine, One Baylor Plaza, Houston, Texas 77030.

TABLE I. Effects of inhibitors on proteolytic activity using peptide substrates.*

| Inhibitor† | Specificity | % Inhibition | | |
|---|---|---|---|---|
| | | Z-Phe-Arg-AFC | Z-Gly-Gly-Arg-AFC | Arg-AFC |
| PMSF (2 mM) | Serine | 0 | 2 | 25 |
| Leupeptin (4 μg/ml) | Serine/cysteine | 98 | 2 | 0 |
| Ep459 (10 μM) | Cysteine | 98 | 0 | 0 |
| Pepstatin (20 μg/ml) | Aspartic | 24 | 49 | 47 |
| EDTA (5 mM) | Metallo | 22 | 99 | 91 |
| 1,10 Phenanthroline (5 mM) | Metallo | 3 | 100 | 91 |

* Data are the mean percent inhibition (at least 2 experiments each done in duplicate) of proteolytic activity as measured by production of 7-amino-4-trifluoromethylcoumarin (AFC) in the presence and absence of the protease inhibitors at final concentrations noted. Z-Phe-Arg-AFC was assayed in acid buffer in the presence of 5 mM cysteine. Arg-AFC and Z-Gly-Gly-Arg-AFC were assayed in neutral buffer.
† PMSF, phenylmethylsulfonyl fluoride; EDTA, disodium ethylenediaminotetraacetate.

succinyl-Ala-Ala-Pro-Met-AFC, and methoxysuccinyl-Ala-Ala-Pro-Val-AFC (serine proteases); Arg-AFC (metallo and cysteine proteases); and Z-Arg-Gly-Phe-Phe-Pro-AFC and Z-Arg-Gly-Phe-Phe-Leu-AFC (aspartic proteases). Assay buffers were 100 mM PBS, pH 7.5 (neutral buffer), or 100 mM sodium citrate, pH 4.9 (acid buffer). To detect the thiol-enhancement characteristic of cysteine proteases, initial assays were performed in a buffer with or without 5 mM cysteine. Peptidase activity was assayed fluorometrically as nanomoles of free AFC produced upon cleavage of the substrate after incubation with S1 (1 hr and overnight at 37 C), as previously described (Dresden et al., 1985; Zerda et al., 1988). Because aspartic proteases normally cleave between 2 hydrophobic amino acids (e.g., Phe-Phe) (reviewed by Barrett [1986]), the substrates Z-Arg-Gly-Phe-Phe-Pro-AFC and Z-Arg-Gly-Phe-Phe-Leu-AFC are expected to yield Phe-Pro-AFC and Phe-Leu-AFC, respectively. A second incubation with an aminopeptidase is required to produce free AFC. We tested for *T. solium* aspartic protease activity by overnight incubation with S1 and the peptide substrates, followed by neutralization and incubation with leucine aminopeptidase (Sigma Chemical Company, St. Louis, Missouri) (37 C, 2 hr) prior to assaying for AFC production. Controls included measurements with S1 immediately prior to incubation with aminopeptidase and measurements with aminopeptidase and no S1.

### Protease assays with protein substrates

Acid or neutral S1 was diluted in the assay buffer at the same pH (1:50 final dilution). Human albumin and human hemoglobin (final concentration 66.7 μg/ml) were added to S1. After overnight incubation at 37 C, the proteins were boiled in sodium dodecyl sulfate (SDS) sample buffer containing 2-mercaptoethanol and separated by SDS-polyacrylamide gel electrophoresis (17.5% acrylamide for hemoglobin and 10–20% gradient of acrylamide for albumin). The gels were stained with silver (Merril et al., 1981). Results were quantified by scanning densitometry.

Immunoglobulin G (IgG) was purified from human serum using a protein A disc (Fischer Scientific, Pittsburgh, Pennsylvania). The purified IgG was biotinylated with *N*-hydroxysuccinimido-biotin as previously described (Guesdon et al., 1979). For protease assays, biotinylated IgG was separated from unincorporated biotin using Sephadex G-25. Biotinylated IgG (3 μg) was incubated with 50 μl of either acid or neutral S1 and brought to 75 μl with assay buffer at the same pH. After incubation (overnight, 37 C), the samples were boiled in SDS-sample buffer, and separated by SDS-PAGE under reducing conditions (12.5% acrylamide, 10 μl/lane). The proteins were blotted onto nitrocellulose. The nitrocellulose was blocked with PBS containing 0.3% Tween-20, washed extensively with PBS containing 0.1% Tween, incubated with horseradish peroxidase-conjugated streptavidin (Zymed, San Francisco, California), washed, and visualized by incubation with 4-chloronaphthol and diaminobenzidine.

### Protease inhibitors

Protease inhibitors were obtained from Sigma Chemical Company unless otherwise noted. Working solutions were made by diluting stock solutions prepared as follows: phenylmethylsulfonyl fluoride (PMSF) (100 mM in acetonitrile), disodium ethylenediaminotetraacetate (EDTA) (100 mM in distilled water), 1,10 phenanthroline (100 mM in acetone), leupeptin (1 mg/ml in distilled water), Ep459 (a gift from Dr. K. Hanada, Taisho Pharmaceutical Co., Tokyo, Japan) (1 mM in dimethyl sulfoxide [DMSO]), and pepstatin (5 mg/ml in DMSO). For inhibitor studies, S1 was preincubated with protease inhibitors (30 min, 37 C) prior to the addition of the substrate. Working concentrations and specificities are shown in Table I.

TABLE II. Representative proteolyic activity measured with synthetic peptide substrates.

| Substrate* | Buffer | 5 mM cysteine | Specific activity (nmoles AFC/hr/mg)† |
|---|---|---|---|
| Z-Phe-Arg-AFC | Acid | – | 0.70 ± 0.22 |
| | Acid | + | 20.42 ± 1.46 |
| Z-Phe-Arg-AFC | Neutral | – | 5.35 ± 0.31 |
| | Neutral | + | 5.77 ± 0.39 |
| Z-Gly-Gly-Arg-AFC | Neutral | – | 2.97 ± 0.19 |
| Arg-AFC | Neutral | – | 21.74 ± 0.82 |

* Substrates are referred to by the 3-letter designation for amino acids and Z for the amino terminal blocking group benzyloxy-carbonyl.
† Specific activity is calculated based on release of 7-amino-4-trifluoromethylcoumarin (AFC) per mg protein in the supernatant fluid per unit time. Values shown are the mean and standard deviation for a representative experiment. Each experiment was repeated at least 4 times.

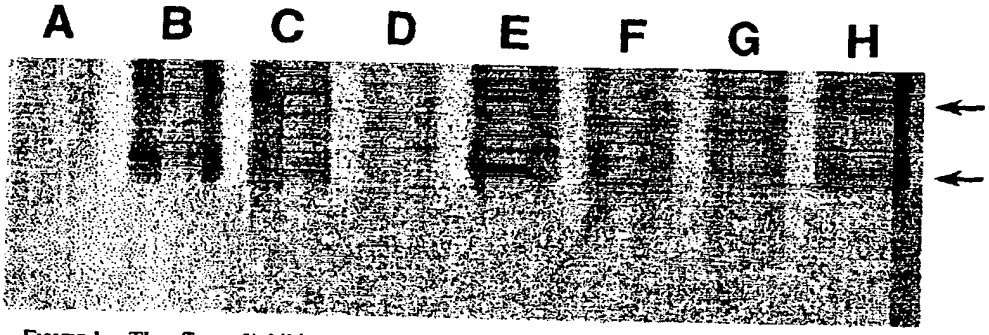


FIGURE 1. The effects of inhibitors on hemoglobin digestion by acid extracts of *Taenia solium* metacestodes. Human hemoglobin was incubated with acid supernatant fluid in acid buffer plus 5 mM cysteine, separated by SDS polyacrylamide gel electrophoresis under reducing conditions, and stained with silver. Lane A, with PMSF; lane B, with EP459; lane C, with pepstatin; lane D, with EDTA; lane E, with PMSF, Ep459, pepstatin, and EDTA; lane F, without inhibitor; lane G, without inhibitor and without cysteine; lane H, control (enzyme and hemoglobin boiled in SDS sample buffer without prior incubation). Solid arrows point to the bands for the $\alpha$- and $\beta$-globin chains.

#### Gel filtration chromatography

Acid and neutral extracts were fractionated by gel filtration chromatography using either Spectragel AcA 54 (Spectrum, Los Angeles, California) (bed volume 1.6 × 48.5 cm, typical flow rate of 30 ml/hr and 2.2-ml fractions) or fast protein liquid chromatography with Superose 12 in an HR10/30 column (Pharmacia, Piscataway, New Jersey) (0.8 ml/min flow rated, 0.5-ml fractions). The columns were equilibrated with assay buffer at the same pH as the extraction buffer. Fractions of acidic extracts were assayed for proteolytic activity using Z-Phe-Arg-AFC, Z-Arg-Gly-Phe-Phe-Leu-AFC, and hemoglobin. Fractions of neutral extracts were assayed using Arg-AFC, Z-Gly-Gly-Arg-AFC, Z-Arg-Gly-Phe-Phe-Pro-AFC, and hemoglobin.

## RESULTS

#### Protease activities

We assayed acid extracts of *T. solium* metacestodes for proteolytic activity using 9 synthetic peptide substrates and 3 protein substrates. Among the peptide substrates, significant activity was detected only with Z-Phe-Arg-AFC, Arg-AFC, and Z-Gly-Gly-Arg-AFC (Table II). Minor activity was detected with Z-Arg-Gly-Phe-Phe-Leu-AFC and Z-Arg-Gly-Phe-Phe-Pro-AFC. The proteolytic activity detected with Arg-AFC and Z-Gly-Gly-Arg-AFC was destroyed completely by a single cycle of freezing and thawing. The enzyme(s) cleaving Z-Phe-Arg-AFC was not destroyed by freezing.

Human proteins albumin, hemoglobin, and IgG also were tested as substrates. No activity was detected with albumin. Both hemoglobin and IgG were markedly degraded at acidic pH (Figs. 1, 2). Only the hemoglobin digestion was enhanced minimally by cysteine. At neutral pH, hemoglobin was cleaved, but less prominently than at acidic pH. IgG degradation only was detected at acidic pH (Fig. 2). Proteolysis was limited to the heavy-chain band (Fig. 2). A new band appeared at 28 kDa. IgG-degrading activity was not enhanced by the addition of cysteine.

Enzyme activities were classified by incubating S1 with inhibitors of known specificity prior to assaying for protease activity (Table II; Figs. 1, 2). The major activities were further characterized by molecular sieve chromatography (Figs. 3–5).

#### Metalloprotease activity

We identified metalloprotease activity by using chelating agents as inhibitors. Both the neutral aminopeptidase activity detected with Arg-AFC and the endopeptidase activity detected with Z-Gly-Gly-Arg-AFC were inhibited completely by EDTA and 1,10 phenanthroline (Table I). Partial inhibition of Z-Gly-Gly-Arg-AFC cleavage was noted consistently with pepstatin. Hemoglobin degradation at neutral pH was also inhibited by EDTA but not significantly by pepstatin, Ep459, or PMSF.

Upon molecular sieve chromatography, hydrolytic activity detected with both Arg-AFC and Z-Gly-Gly-Arg-AFC eluted as a peak with a molecular weight of 104 kDa (Fig. 3). Hemoglobin also was degraded at neutral pH by the fractions that hydrolyzed Arg-AFC activity.

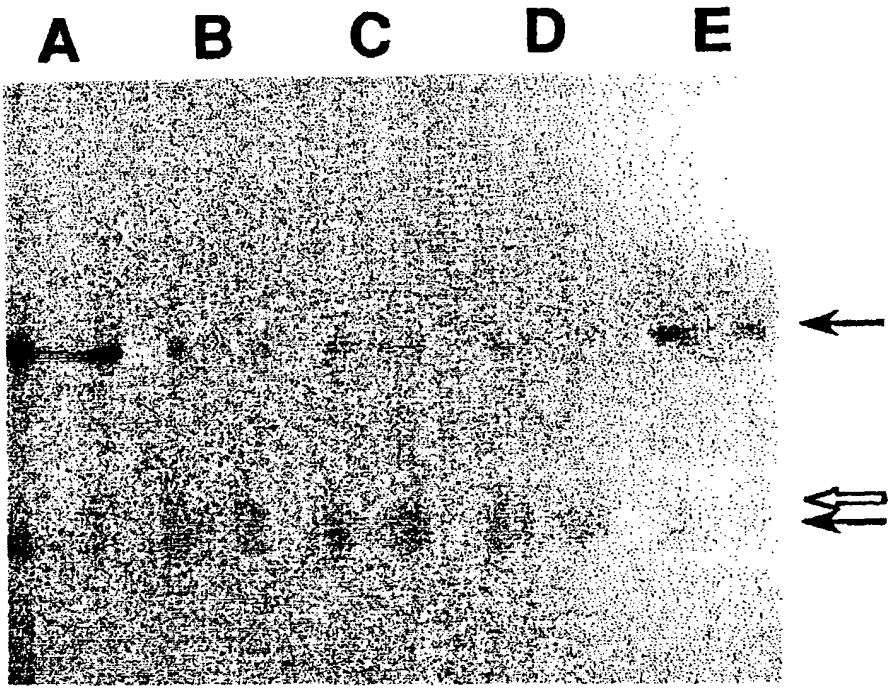


FIGURE 2. The effects of inhibitors on IgG digestion by acid extracts of *Taenia solium* metacestodes. Biotinylated IgG was incubated with acid extracts of *T. solium* metacestodes and inhibitors, separated by SDS polyacrylamide gel electrophoresis under reducing conditions, blotted onto nitrocellulose, and visualized with peroxidase-conjugated streptavidin. Lane A, with pepstatin; lane B, with PMSF, EDTA, and Ep459; lane C, without inhibitor; lane D, without inhibitor and without cysteine; lane E, control (biotinylated IgG and enzyme boiled in SDS sample buffer without prior incubation). Solid arrows point to the heavy-chain and light-chain bands. Open arrow points to the major proteolytic fragment.

#### Cysteine protease activity

Cysteine protease activity was identified by thiol enhancement and by inhibition by leupeptin and Ep459. Hydrolysis of Z-Phe-Arg-AFC was enhanced by the addition of cysteine and was greater at acidic pH (Table II). The acidic hydrolytic activity was blocked completely by inhibitors of cysteine proteases (i.e., leupeptin and Ep459) but not by inhibitors of other classes of enzymes (Table I). In contrast to the activity noted at acid pH, cleavage of Z-Phe-Arg-AFC at neutral pH was not enhanced by cysteine and was inhibited only partially (50%) by leupeptin or Ep459. Partial inhibition was also noted with pepstatin and chelating agents, suggesting that the overall activity is due to enzymes of multiple classes. Hemoglobin degradation at acidic pH was enhanced partially by cysteine and was inhibited partially (46%) by Ep459 (Fig. 1).

Upon gel filtration chromatography, proteolytic activity detected with Z-Phe-Arg-AFC eluted in a broad peak in fractions corresponding to a molecular weight of about 32 kDa (Fig. 4).

#### Aspartic protease activity

Aspartic protease activity was identified using the class-specific inhibitor pepstatin. There was 92% inhibition of hemoglobin degradation at acidic pH by pepstatin (Fig. 1). A pool of inhibitors (including Ep459 and pepstatin) was required to inhibit hemoglobin degradation totally (Fig. 1). IgG degradation was inhibited totally by pepstatin (Fig. 2). No inhibition of IgG degradation was noted with Ep459, EDTA, or PMSF individually or as a pool (Fig. 2).

Chromatographic fractions of acid S1 were assayed for proteinase activity with hemoglobin. Protein degradation was noted in a broad peak

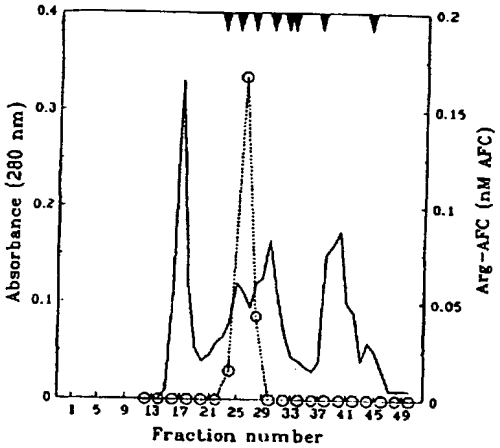


FIGURE 3. Gel filtration chromatography of neutral extracts of *Taenia solium* metacestodes. Neutral extracts were separated by fast protein liquid chromatography gel filtration using a Superose 12 column. Fractions were checked for optical density at 280 nm (solid line) and assayed for proteolytic activity with Arg-AFC (dotted line). Arrowheads mark the elution volume of calibration standards (from left to right 443, 200, 66.3, 36.8, 25, 13.3, 3.9, and 0.2 kDa). AFC, 7-amino-4-trifluoromethylcoumarin.

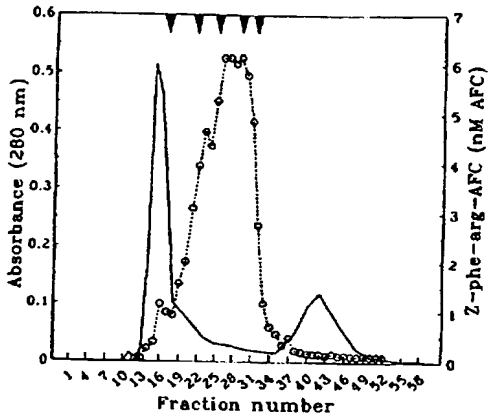


FIGURE 4. Gel filtration chromatography of acid extracts of *Taenia solium* metacestodes. Acid extracts were separated by gel filtration chromatography with AcA 54. Fractions were checked for absorbance at 280 nm (solid line) and assayed for proteolytic activity with Z-Phe-Arg-AFC (dotted lines). Arrowheads mark the elution volume of calibration standards (from left to right 200, 66.3, 36.8, 25, and 13.3 kDa). AFC, 7-amino-4-trifluoromethylcoumarin.

with maximal activity noted at about 90 kDa (Fig. 5). In the presence of Ep459 alone, activity was also maximal in these high molecular weight fractions. When fractions were assayed in the presence of pepstatin alone, there was complete inhibition of activity in the 90-kDa peak; residual activity was maximal at about 32 kDa (previously identified as cysteine protease activity). IgG also was cleaved by those fractions with pepstatin-inhibitable hemoglobinase activity.

### Serine protease activity

We attempted to identify serine protease activity using 6 peptide substrates normally cleaved by serine proteases. In addition, we studied the effects of serine protease inhibitors (PMSF and leupeptin) on digestion of peptide and protein substrates. No activity was detected with Z-Arg-AFC, succinyl-Ala-Ala-Pro-Phe-AFC, methoxysuccinyl-Ala-Ala-Pro-Met-AFC, or methoxysuccinyl-Ala-Ala-Pro-Val-AFC. The activity detected with Z-Gly-Gly-Arg-AFC was inhibited by chelating agents but not PMSF or leupeptin. Hydrolysis of Z-Phe-Arg-AFC was inhibited by leupeptin. However, hydrolysis was inhibited by Ep459 and not with PMSF. Neither hemoglobin nor IgG degradation was inhibited with PMSF. Thus, we did not detect any serine protease activity.

### Unclassified activity

Proteolytic activity was also noted with Z-Arg-Gly-Phe-Phe-Leu-AFC (6.01 ± 0.15 nmoles

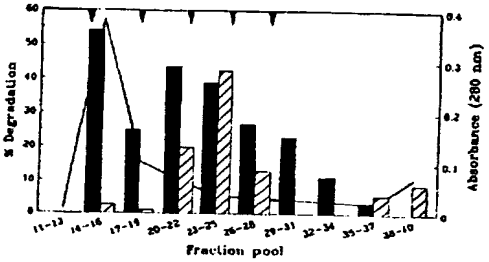


FIGURE 5. Gel filtration chromatography of acid extracts of *Taenia solium* metacestodes. Acid extracts were separated by gel filtration chromatography with AcA 54. Pooled fractions were assayed for proteolytic activity with hemoglobin (solid bars). Hemoglobin digestion was also examined in the presence of pepstatin (hatched bars). The superimposed solid line and arrowheads illustrate the chromatographic run from which the pooled fractions were derived. The line represents the absorbance at 280 nm of the respective fraction pools. Arrowheads mark the elution volume of calibration standards (from left to right 200, 66.3, 36.8, 25, and 13.3 kDa).

AFC/mg with cysteine and 5.06 ± 0.32 nmoles AFC/mg without cysteine at acidic pH). Cleavage of Z-Arg-Gly-Phe-Phe-Leu-AFC was inhibited only partially by leupeptin and Ep459 and not inhibited by other inhibitors. Z-Arg-Gly-Phe-Phe-Pro-AFC was cleaved by S1 at neutral pH (3.74 ± 0.09 nmoles AFC/mg with cysteine and 1.90 ± 0.15 nmoles AFC/mg without cysteine). For Z-Arg-Gly-Phe-Phe-Pro-AFC, activity was partially inhibited by pepstatin and Ep459. Inhibition with chelating agents could not be assayed with these substrates because the activity was detected only in the presence of an exogenous metalloenzyme.

## DISCUSSION

At neutral pH, we have demonstrated evidence for proteolytic activity with hemoglobin and the synthetic substrates Arg-AFC and Z-Gly-Gly-Arg-AFC. All of this activity was inhibited by chelating agents, suggesting that this activity is due to metalloprotease(s). Both the aminopeptidase and endopeptidase activity were in the same fractions from gel filtration columns, suggesting that a single enzyme may be involved. Although it is unusual for a single enzyme to demonstrate both activities, cathepsin H, a cysteine protease, has been previously shown to do so (Mason, 1989).

Activity demonstrated with Z-Phe-Arg-AFC is enhanced by thiols, inhibited by Ep459 and leupeptin, and has an acid pH optimum. These properties are characteristic of cysteine proteases. The molecular weight by gel filtration is about 32 kDa, similar to the size of the cathepsin family of cysteine proteases (Barrett, 1986). The activity eluted in a broad band, suggesting that several enzymes may be involved. At present, the physiologic role of this activity is not known. Similar cysteine proteases from schistosomes digest hemoglobin, a major source of amino acids for the adult worms (Zerda et al., 1988).

Albumin and immunoglobulin are the major components of serum and the major host proteins in *Taenia* cyst fluid (Kunz et al., 1989). No albumin degradation was noted at either acid or neutral pH. This is consistent with observations by others on the stability of albumin within the metacestode cyst fluid (Hayunga et al., 1989). In contrast, we have demonstrated that human immunoglobulin is degraded readily by an enzyme present in S1. Both IgG and hemoglobin were cleaved at acid pH predominantly by enzymes that could be inhibited by pepstatin, a specific inhibitor of aspartic proteases. Maximal activity was noted in gel filtration fractions with a molecular weight of about 90 kDa.

Host immunoglobulins are found both bound to the metacestode surface and within the cyst fluid (Correa et al., 1986; Craig, 1988; McManus and Lamsam, 1990). Preliminary evidence suggests Fc receptorlike uptake of host immunoglobulin (Willms et al., 1982; Craig, 1988; Hayunga et al., 1989), which may be digested as a source of amino acids (Damian, 1987). By preferential degradation of the heavy chain, the aspartic proteinase may cleave the complement-fixing and opsonic portions of the IgG. Cleaved antibody could function to block epitopes that might otherwise lead to host immune destruction of the metacestode, as has been suggested in vivo for "blocking" antibody (Rickard, 1974). Although numerous enzymes can degrade IgG in vitro, using *T. solium* metacestodes, we demonstrated degradation only by an aspartic protease(s).

We identified IgG degradation only at acidic pH. As IgG is normally in locations with neutral pH, the physiologic significance of this finding is unclear. Pinocytotic vesicles appear to be the major mechanism of transport of host proteins into the cyst fluid (Threadgold and Dunn, 1983, 1984). Because some of these vesicles fuse with lysosomes, it is conceivable that lysosomal proteases might be involved in processing host proteins that are ultimately transported into the cyst fluid.

No serine protease activity was demonstrated. *Taenia* species metacestodes secrete taeniastatin, a protease inhibitor with antitrypsin and antichymotrypsin activities (Leid et al., 1984). It is possible that serine protease activity was inhibited by endogenous inhibitors such as taeniastatin. Alternatively, enzymes may have been destroyed during lyophilization. Another explanation is that the metacestode uses only enzymes of other classes. The endogenous inhibitor, taeniastatin, then could modulate host proteases without affecting its own enzymes.

We used whole parasites as the source of enzymes. Host components are a major constituent of the cyst fluid (Hayunga et al., 1989; Kunz et al., 1989) and host proteins often are detected on the membranes of metacestodes (Correa et al., 1986; Craig, 1988; McManus and Lamsam, 1990). Thus, it is impossible at this stage to be certain that these enzymes are of parasite and not host origin. However, all 3 proteolytic activities were found in cyst fluid and cyst walls as well as in homogenates, suggesting that the enzymes are of parasite origin. Whether of host or

parasite origin, clearly proteolytically active enzymes are present within the parasite and likely play a role in parasite physiology. Future studies involving enzyme purification and structural studies should help clarify the origin of these proteases.

## ACKNOWLEDGMENTS

This work was supported in part by the Lederle Pharmaceuticals Young Investigator Award in Vaccine Development of the Infectious Diseases Society of America to A.C.W. We thank Cynthia Chappell for many helpful suggestions.

## LITERATURE CITED

AURIAULT, C., M. JOSEPH, J. P. DESSAINT, AND A. CAPRON. 1980. Inactivation of rat macrophages by peptides resulting from cleavage of IgG by *Schistosoma* larvae proteases. Immunology Letters 2: 135–139.

———, M. A. OUAISSI, G. TORPIER, H. EISEN, AND A. CAPRON. 1981. Proteolytic cleavage of IgG bound to the Fc receptor of *Schistosoma mansoni* schistosomula. Parasite Immunology 3: 33–44.

BARRETT, A. J. 1986. An introduction to the proteinases. *In* Proteinase inhibitors, A. J. Barrett and G. Salvesen (eds.). Elsevier Science Publishers BV, New York, p. 3–22.

BRADFORD, M. 1976. A rapid and sensitive method for the quantitation of protein–dye binding. Analytical Biochemistry 72: 248–254.

CORREA, D., C. GORODESKY, L. CASTRO, T. RABIELA, AND A. FLISSER. 1986. Detection of MHC products on the surface of *Taenia solium* cysticerci from humans. Revista Latino-americana de Microbiologia 28: 363–371.

CRAIG, P. S. 1988. Surface-associated proteins and host IgG on early and late metacestode stages of *Taenia pisiformis*. Parasite Immunology 10: 243–254.

DAMIAN, R. T. 1987. The exploitation of host immune responses by parasites. Journal of Parasitology 73: 3–13.

DIXON, H. B. F., AND F. M. LIPSCOMB. 1961. Cysticercosis: An analysis and follow-up of 450 cases. Her Majesty's Stationary Service, London, 58 p.

DRESDEN, M. H., A. A. REGE, AND K. D. MURRELL. 1985. *Strongyloides ransomi*: Proteolytic enzymes from larvae. Experimental Parasitology 59: 257–263.

EARNEST, M. P., L. B. RELLER, C. M. FILLEY, AND A. J. GREK. 1987. Neurocysticercosis in the United States: 35 cases and a review. Reviews of Infectious Diseases 9: 961–979.

FLISSER, A. 1988. Neurocysticercosis in Mexico. Parasitology Today 4: 131–137.

GUESDON, J.-L., T. TERNYNCK, AND S. AVRAMEAS. 1979. The use of avidin–biotin interaction in immunoenzymatic techniques. Journal of Histochemistry and Cytochemistry 27: 1131–1139.

HAYUNGA, E. G., M. P. SUMNER, AND T. LETONJA. 1989. Evidence for selective incorporation of host immunoglobulin by strobilocerci of *Taenia taeniaeformis*. Journal of Parasitology 75: 638–642.

HOTEZ, P. J., AND A. CERAMI. 1983. Secretion of a proteolytic anticoagulant by *Ancylostoma* hookworms. Journal of Experimental Medicine 157: 1594–1603.

———, J. HAGGERTY, J. HAWDON, L. MILSTONE, H. R. GAMBLE, G. SCHAD, AND F. RICHARDS. 1990. Metalloproteases of infective *Ancylostoma* hookworm larvae and their possible function in tissue invasion and ecdysis. Infection and Immunity 58: 3883–3892.

———, L. N. TRANG, J. H. MCKERROW, AND A. CERAMI. 1985. Isolation and characterization of a proteolytic enzyme from the adult hookworm *Ancylostoma caninum*. Journal of Biological Chemistry 260: 7343–7348.

KUNZ, J., B. KALINNA, V. WATSCHKE, AND E. GEYER. 1989. *Taenia crassiceps*, metacestode vesicular fluid antigens shared with *Taenia solium* larval stage and reactive with serum antibodies from patients with neurocysticercosis. Zentralblat für Bakteriologie 271: 510–520.

LEID, R. W., C. M. SUQUET, AND L. E. PERRYMAN. 1984. Inhibition of antigen- and lectin-induced proliferation of rat spleen cells by a *Taenia taeniaeformis* proteinase inhibitor. Clinical and Experimental Immunology 57: 187–194.

MASON, R. W. 1989. Interaction of lysosomal cysteine proteinases with α2-macroglobulin: Conclusive evidence for endopeptidase activities of cathepsins B and H. Archives of Biochemistry and Biophysics 283: 367–374.

MCKERROW, J. H. 1989. Parasite proteases. Experimental Parasitology 68: 111–115.

MCMANUS, D. P., AND S. LAMSAM. 1990. *Taenia crassiceps* surface immunoglobulin: Parasite- or host-derived? Parasitology 101: 127–137.

MERRIL, C. R., D. GOLDMAN, S. A. SEDMAN, AND M. H. EBERT. 1981. Ultrasensitive stain for proteins in polyacrylamide gels shows regional variation in cerebrospinal fluid proteins. Science 211: 1437–1438.

RICKARD, M. D. 1974. Hypothesis for the long term survival of *Taenia pisiformis* cysticerci in rabbits. Zentralblatt für Parasitenkunde 44: 203–209.

THREADGOLD, L. T., AND J. DUNN. 1983. *Taenia crassiceps*: Regional variation in ultrastructure and evidence of endocytosis in the cysticercus tegument. Experimental Parasitology 55: 121–131.

———, AND ———. 1984. *Taenia crassiceps*: Basic mechanisms of endocytosis in the cysticercus. Experimental Parasitology 58: 263–269.

VERWAERDE, C., C. AURIAULT, J. L. NEYRINCK, AND A. CAPRON. 1988. Properties of serine proteases of *Schistosoma mansoni* schistosomula involved in the regulation of IgE synthesis. Scandinavian Journal of Immunology 27: 17–24.

WILLMS, K., M. T. MERCHANT, S. DIAZ, AND L. ARCOS. 1982. Host–parasite interface in the metacestode of *Taenia solium*. *In* Cysticercosis: Present state of knowledge and perspectives, A. Flisser, K. Willms, J. P. LaClette, and C. Larralde, (eds.). Academic Press, New York, p. 397–411.

ZERDA, K. S., M. H. DRESDEN, AND C. L. CHAPPELL. 1988. *Schistosoma mansoni*: Expression and role of cysteine proteases in developing schistosomula. Experimental Parasitology 67: 238–246.